EFFICACY TEST PROTOCOL

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1 TITLE: TEST OF PERSONAL INSECT REPELLENTS

2 PROTOCOL NUMBER:

EMD–004

3 SPONSOR:

EMD Chemicals, Inc.

3.1 Address:

7 Skyline Drive, Rona–Cosmetic Business Unit
Hawthorne, NY 10532 USA

4 PROTOCOL OBJECTIVE:

4.1 Type of Protocol:

This protocol will indicate the specific methods to be used and direct the conduct of the Study EMD–004. This protocol functions with the general Carroll-Loye Protocol C-L–001, entitled "Protocol for Tests of Personal

Insect Repellents". That protocol presents the domain of and universal instructions for conducting tests of this class, as required by the California Environmental Protection Agency. That and this protocol were developed by Dr. Scott Carroll, Director of Research, Carroll-Loye Biological Research.

The study will be conducted at locales in nature with mosquitoes.

5 STUDY OBJECTIVE:

5.1 Objective of Research

To test the repellent characteristics of the test materials, with efficacy measured in terms of percent reduction in the rate of alightments ('lites') as well as "Complete Protection Time" for comparative purposes.

5.2 Rationale and Main Endpoint:

The main endpoint of this study will be the conclusion mosquito and tick repellent efficacy tests conducted in the field of an IR3535-based topical repellent.

5.3 Standards Applied:

U. S. EPA Good Laboratory Practice Regulations (40 CFR 160).
California State EPA study monitoring.

6 INVESTIGATIONAL AND TEST MATERIAL CONTROL:

6.1 Test Substance:

6.1.1 Description of the Test Substance

Formulations containing EMD's proprietary IR3535-based repellent will be tested. IR3535 is a US/EPA-registered repellent active ingredient, Ethylbutylacetylamino-propionate. It is the active ingredient in numerous registered commercial personal insect repellents marketed worldwide, including the US/EPA-registered Avon Bug Guard line. The three test formulations are Lotion WV29-01-9N (lot # M17345), Aerosol EUS26-16-9N (lot # M17346), and Spray EUS26-

15-9N (lot # M17279). Details of the test formulations are in the Appendix.

6.1.2 Dosage Form:

Liquid applied to exposed skin.

6.1.3 Dose:

The substance will be applied to a defined test site on the subjects' lower arms and/or lower legs at a concentration of approximately 1.6 micrograms per cm^2 of skin surface area (to give 1.0 mg per 600 cm^2 of skin surface area.

6.2. Positive Control (Standard):

6.2.1 Description of the Control Substance

An industry-standard EPA-registered commercial arthropod repellent, such as Deep Woods OFF!™ (S. C. Johnson and Sons, Inc., Racine Wisconsin, USA) will be used as the positive control. It will be purchased over-the-counter prior to the test. Its market formulation is approximately 20% DEET.

6.2.2 Dosage Form:

Lotion applied to exposed skin.

6.2.3 Dose:

The standard will be applied to a defined test site on the subjects' lower arms and legs at a concentration of 1.0 mg per 600 cm^2, based on the manufacturer's test protocol.

6.3. Negative Controls:

6.3.1 Description of the Negative Controls

The negative control is untreated.

6.4 Test Arthropod Species:

Testing will be conducted with *Aedes/Ochlerotatus* and *Culex* mosquitoes.

7 STUDY SCHEDULE:

7.1 Proposed Date of Initiation:

TBD, within one year of IRB approval.

7.2 Schedule of Events:

Test day	Date	Activities
-10—2	TBD	Begin subject recruitment. Introduce subjects to test plan and procedures; explain payment; review subject rights and consent forms; option to sign consent forms in order to participate; measure limb surface areas; calculate individual dosages.
1	TBD	Prepare individual dosages for application. Meet with subjects to review day plan and safety procedures. Travel to field site. Review safety and data collection procedures. Administer repellent, commence data collection. Monitor subject safety, comfort, comportment, compliance with data collection protocol.

7.3 Proposed Date of Completion:

Experimental Completion Date (Test Day 1): TBD.
Final Report Completion Date: TBD.

8 STUDY DESIGN:

8.1 Treatment Groups:

There are three treatment groups, namely 1) a test product treatment group, in which there are three formulations , 2) a positive standard (DEET) group, and 3) an untreated negative control group.

8.2 Experimental Design:

The experiment will be treated as a partially randomized, experimenter and subject-blinded trial. However, control subjects will be chosen only from among individuals that are experienced in field biology or entomology.

8.3 Randomization Procedures:

8.3.1 Allocation of subjects to treatment groups:

Subjects will be assigned to the treatment groups on the basis of a randomly assigned subject number. Subjects will be assigned treatment based on subject number from the treatment allocation table, which follows. Treatments will be balanced between arms and legs.

8.3.2 Treatment allocation table:

Materials will be distributed among subjects as tabulated below. There will in addition be one positive control and one negative control. For blinding and logistic concerns, the actual distribution will differ inconsequentially from this.

Subject	Lotion	Pump	Aerosol	DEET	Untreated
1	Left limb				
2	Right limb				
3	Left limb				
4	Right limb				
5	Left limb				
6	Right limb				
7		Left limb			
8		Right limb			

9		Left limb			
10		Right limb			
11		Left limb			
12		Right limb			
13			Left limb		
14			Right limb		
15			Left limb		
16			Right limb		
17			Left limb		
18			Right limb		
19				Left limb	
20					Right limb

8.4. Conditional Boundaries or Limits of Study

8.4.1. Ambient liting Pressure, mosquitoes:

A mean study alighting ('lite') rate of ≥ 5 bites per untreated (negative control) lower leg or lower arm per 5 minutes is required. Ambient liting pressure is measured from continuous exposure during 5-minute measurement intervals, beginning at the onset of data collection. If ambient liting pressure is too great for the comfort or safety of the negative control subjects, these data may be collected based on 5-minute exposures conducted every fifteen minutes, beginning at the onset of data collection. Control subjects use aspirators to remove alighting mosquitoes before biting commences.

Mean liting rate is calculated as the number of lites received per period of exposure.

9 STUDY PROCEDURES:

9.1 Test Subjects:

9.1.1 Inclusion criteria:

9.1.1.1	Age:	At least 18 yrs
9.1.1.2	Sex:	Male/female

9.1.1.3 Race: Any race
9.1.1.4 Written consent (see 9.4, below).

9.1.2 Exclusion criteria:

9.1.2.1 Known to be to be hypersensitive to mosquito bites.
9.1.2.2 Known to be to be sensitive to any of the test product ingredients.
9.1.2.3 Poor physical condition.
9.1.2.4 Unwilling to submit to brief query about personal condition.
9.1.2.5 Not able to write, and speak English at approximately the University of California college level.
9.1.2.6 Unwilling to refrain from use of alcoholic beverages or smoking during the test.
9.1.2.7 Known to be pregnant or lactating. Pregnancy will be self-checked by each female volunteer on the morning of the repellent test using an OTC test kit provided by the Study Director. Results of each such test will be immediately verified by direct inspection by a female technician trained to make that assessment. Only volunteers scored as nonpregnant will be allowed to participate.

9.1.3 Number of Subjects:

Approximately 6-10 per treatment group, and 1-2 per control group.

9.2 Blinding of Study:

9.2.1. Extent of the Blinding:

Subjects will be blinded to the treatments they receive.
Study Director will be blinded to the identity of all test substances until the conclusion of data evaluation.

9.2.2 Blinding Methods:

Data capture forms will be coded with respect to treatment, so that personnel recording data (subjects) will not be aware of the treatments that they have received.

9.3. Study Material Administration:

Study Materials will be administered to each subject by Carroll-Loye technicians. Test products will be applied volumetrically. Test sites are first cleansed with water and a fragrance free detergent soap, rinsed with an isopropanol/water solution, and then towel dried. Test products are applied to the test site from a syringe or micropipette; they are spread on the site as evenly as possible with two fingertips in a surgical glove, using a light rubbing motion.

9.4 Subject Consent:

Written subject consent (Carroll-Loye California EPA approved Participant Consent Form) is an inclusion criterion.

10 SPECIFICATION OF THE VARIABLES:

10.1 Variable to be Measured:

Number of mosquito lites (measured as number of mosquitoes alighting on the treated surface (termed 'lites'), with liting mosquitoes immediately aspirated, before biting).

10.1.1 When Variable will be Assessed:

Subjects will record any lites as they occur. Data are recorded in 5 minute blocks. Exposure is continuous. The time at which the application of a treatment is completed is recorded as t_0 ('time zero'). There may be a delay of no more than 10 minutes after treatment until exposure begins.

10.1.2 Forms for Retention of Source Data:

Lite data will be recorded on a data form.

11 DATA ANALYSIS:

11.1 Experimental Unit:

The individual subject will be the experimental unit.

11.2 Replicates per Treatment:

There will be a minimum of six subjects treated with the each test repellent, one serving as untreated controls, and one testing the standard.

11.3 Statistical Methodology:

Field tests are conducted with large populations of arthropods. This permits the analysis of the replicates (data by subject) as independent values. The hypothesis to be tested is that the treatment will significantly reduce the number of mosquitoes alighting on treated versus untreated skin. The likely prevalence of 0 values and low values (1 or 2) indicates the use of contingency analyses (e.g., Chi-square). Alpha is 0.05. Analyses may compare subsets of the data (e.g., first two hours) in addition to the entire data set.

Complete protection time (CPT) is measured as the length of time from initial application to the first confirmed failure. A confirmed failure is a lite followed by another lite within 30 minutes. For example, a lite at 10 minutes followed by another at 55 minutes is not confirmed, but a third lite at 65 minutes would confirm that at 55 minutes, giving a CPT of 55 min.

Descriptive statistics include the mean and standard deviation of complete protection times, failure rates and the numbers of failures. In addition, two general classes of statistical analyses may be applied. The first class compares the efficacy of the prototypes to the control and the comparison article(s). The percentage reduction in mean total lites is calculated as [1 -

Mean comparator/Mean Untreated]100. The second class tests for uniformity over time in each treatment group to test the hypothesis that repellency does not decline with time. The distribution of scores will determine whether parametric statistical tests (e.g. analysis of variance and regression) or non-parametric statistical tests (e.g. the Kruskal-Wallis test and the Spearman rank correlation) are most appropriate for these analyses. The results of these analyses will be discussed with reference to the efficacy of each product, to the feeding biology of the pest species and to the context of product application.

12 STUDY LOCATION(S):

Field sites are in or adjacent to the Central Valley of California, and the Florida Keys (depending on season).

13 PERSONNEL:

13.1 Investigator (Study Director):

13.1.1 Address

Dr. Scott Carroll
Carroll—Loye Biological Research
711 Oak Avenue
Davis, CA 95616

13.1.2 Telephone

530-297-6080
530-297-6081 (Facsimile)

13.1.3 Training and experience of investigator

CV on file with sponsor

13.2 Study Monitor:

Dan Giambattisto

13.2.1 Address

EMD Chemicals, Inc.
7 Skyline Drive
Rona–Cosmetic Business Unit
Hawthorne, NY 10532 USA

13.3 Quality Assurance Unit:

Dr. Jenella Loye

13.3.1 Address

Carroll—Loye Biological Research
711 Oak Avenue
Davis, CA 95616

13.3.2 Telephone

530-297-6080
530-297-6081 (Facsimile)

13.1.3 Training and experience of QAU

CV on file with sponsor

14 AMENDMENT/DEVIATIONS TO THE PROTOCOL:

Protocol amendments or deviations will be reviewed by the Study Monitor and the Study Director. The amendments or deviations will be documented in the Study Director's final report. Documentation will include a description of the change, the reason for the change and the effect of the change on the experiment.

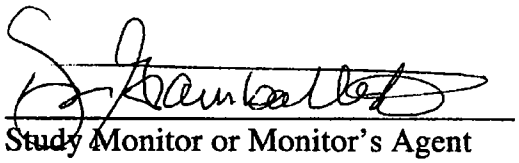
15 PROTOCOL APPROVAL SIGNATURES:

Note: Signing by appropriate persons of a "Study Initiation Protocol" that references the study described herein constitutes the completion of this Protocol Approval page (Section 15).

23 February 2006

Scott P. Carroll, Ph.D.
Study Director

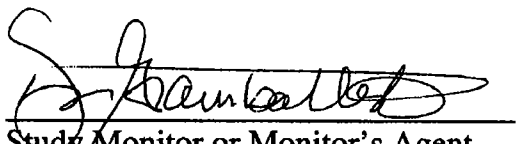
Date



13 April 2006

Study Monitor or Monitor's Agent
Dan Giambattisto

Date



13 April 2006

Study Monitor or Monitor's Agent

Date

Appendix. Test repellent formulations.

Insect Repellent Spray with IR3535®
(EUS26-15)

Ingredients	INCI	[%]	CAS No.	EPA Inert List
Phase A				
IR3535®	Ethyl Butylacetylamino-propionate	20.00	52304-36-6	**Active Ingredient**
Carbowax 400 /Union Carbide	Polyethylene glycol 400	5.00	25322-68-3	4B
Arlamol E	PEG-15 Stearyl Ether	1.00	25231-21-4	4B
Phase B				
Ethanol SD 40B	Denatured Alcohol	35.00	61116-08-3	4B
Carbowax 1450 /Union Carbide	Polyethylene glycol 1500	4.00	25322-68-3	4B
PVP/VA Copolymer E-735 /ISP	PVP/VA copolymer	2.00	25086-89-9 64-17-5	
Polysorbate 20 / Uniquema	Tween 20	1.50	9005-64-5	4B
Water, demineralized	Aqua (Water)	31.50	7732-18-5	4A

Insect Repellent Aerosol with IR3535®
(EUS26-16)

Ingredients	INCI	[%]	CAS No.	EPA Inert List
Phase A				
IR3535®	Ethyl Butylacetylamino-propionate	20.00	52304-36-6	**Active Ingredient**
Phase B				
Ethanol SD 40B	Denatured Alcohol	21.67	61116-08-3	4B
Propylene glycol / Union carbide	Propylene glycol	4.34	57-55-6	
PVP/VA Copolymer E-735 /ISP	PVP/VA copolymer	1.73	25086-89-9 64-17-5	
Water, demineralized	Aqua (Water)	17.26	7732-18-5	4A
Phase C				
A31, Isobutane /Aeropres	Isobutane	35.00	75-28-5	

Insect Repellent Lotion with IR3535®
(WV29-01)

Ingredient	INCI	(%)
PHASE A		
Water, demineralized	AQUA (WATER)	ad 100
1,3-Butanediol (Merck KGaA)	BUTYLENE GLYCOL	4.00
Titriplex® III (Merck KGaA)	DISODIUM EDTA	0.10
PHASE B1		
Rhodicare-S (Rhodia GmbH)	XANTHAN GUM	0.20
Carbopol ETD 2050 (Noveon)	CARBOMER	0.30
PHASE B2		
Triethanolamine (Merck KGaA)	TRIETHANOLAMINE	0.20
PHASE C		
Arlacel 165 VP (Uniquema)	GLYCERYL STEARATE, PEG-100 STEARATE	3.50
Dow Corning 200 (100cs) (Dow Corning)	DIMETHICONE	0.50
Isopropyl palmitate (Cognis)	ISOPROPYL PALMITATE	4.00
Lanette 16 (Cognis)	CETYL ALCOHOL	1.00
Crodamol STS (Croda)	PPG-3 BENZYL ETHER MYRISTATE	2.00
IR3535®	**ETHYL BUTYLACETYLAMINOPROPIONATE**	**10.00**
Stearic acid (Merck KGaA)	STEARIC ACID	2.00
PHASE D		
Seibel 305 (Seppic)	LAURETH-7, POLYACRYLAMIDE, C13-14 ISOPARAFFIN	1.00
PHASE E		
Triethanolamine (Merck KGaA)	TRIETHANOLAMINE	0.10
PHASE F		
Paragon II/McIntyre	PROPYLENE GLYCOL, DMDM HYDANTOIN, METHYLPARABEN, PROPYLPARABEN	1.00